

U.S. Application No. 09/601,444
Inventor: Esther H. CHANG

AMENDMENTS TO THE CLAIMS

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1. (withdrawn)
 2. (withdrawn)
 3. (withdrawn)
 4. (withdrawn)
 5. (withdrawn)
 6. (withdrawn)
 7. (withdrawn)
 8. (withdrawn)
 9. (withdrawn)
 10. (withdrawn)
 11. (withdrawn)
 12. (withdrawn)

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13. (currently amended) A vector for the systemic delivery of a therapeutic or diagnostic agent to a target cell within a host animal, comprising a complex of a cell-targeting ligand, a cationic liposome comprising a cationic lipid and a neutral or helper lipid, and said agent, wherein the vector has a mean diameter of less than about 100 nm and the ligand is bound directly to the liposome.

14. (original) The vector according to claim 13 having a mean diameter of about 30 to 75 nm.

15. (original) The vector according to claim 13 having a mean diameter of about 50 nm.

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16. (original) The vector according to claim 13 wherein said agent is a nucleic acid.

17. (original) The vector according to claim 13 wherein said agent encodes (a) a protein or (b) an antisense oligonucleotide.

18. (original) The vector according to claim 13 wherein said agent is a nucleic acid encoding wild-type p53.

19. (original) The vector according to claim 13 wherein said ligand is a tumor cell targeting ligand.

20. (original) The vector according to claim 13 wherein said ligand is folate or transferrin.

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21. (original) The vector according to claim 13 wherein said ligand is folate.

22. (original) The vector according to claim 13 wherein said ligand is transferrin.

23. (canceled herein)

24. (original) The vector according to claim 16 wherein said liposome and said nucleic acid are present at a ratio ranging from 0.1-50 nanomoles liposome per 1.0 μ g nucleic acid.

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25. (original) The vector according to claim 24 wherein said ratio ranges from 1.0-24 nanomole liposome per 1.0 μ g nucleic acid.

26. (original) The vector according to claim 24 wherein said ratio ranges from 6-16 nanomoles liposome per 1.0 μ g nucleic acid.

27. (original) The vector according to claim 13 wherein said vector has an acentric structure.

28. (original) The vector according to claim 27 wherein said vector has a solid core.

29. (withdrawn)

30. (withdrawn)

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31. (currently amended) A vector for delivering *in vivo* a therapeutically effective nucleic acid molecule to a tumor-bearing animal, the vector consisting essentially of a complex of a cell-targeting ligand selected from the group consisting of folate and transferrin, a cationic liposome comprising a cationic lipid and a neutral or helper lipid, and a nucleic acid molecule, wherein said vector has a mean diameter of less than about 100 nm and the folate or transferrin ligand is bound directly to said liposome.

32. (original) The vector of claim 31 wherein said nucleic acid molecule encodes wild type p53.

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33. (original) The vector of claim 31 wherein said liposome and said nucleic acid molecule are in a ratio of 0.1-50 nanomole liposome per 1.0 µg nucleic acid.

34. (original) The vector of claim 31 wherein said liposome and said nucleic acid molecule are in a ratio of 1.0-24 nanomole liposome per 1.0 µg nucleic acid.

35. (original) The vector of claim 31 wherein said liposome and said nucleic acid molecule are in a ratio of 6-16 nanomole liposome per 1.0 µg nucleic acid.

36. (original) The vector of claim 31 wherein said vector has an acentric structure.

37. (original) The vector of claim 36 wherein said vector has a solid core.

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38. (currently amended) A pharmaceutical composition comprising a vector according to claim ~~29~~ or 31 in a pharmaceutically acceptable carrier.

39. (withdrawn)

40. (currently amended) A method for providing a therapeutic agent to an animal in need thereof, comprising administering to said animal a therapeutically effective amount of a complex comprising a cell-targeting ligand, a cationic liposome comprising a cationic lipid and a neutral or helper lipid, and said therapeutic agent, wherein said vector has a mean diameter of less than about 100 nm and said ligand is bound directly to said liposome.

41. (original) The method of claim 40 wherein said agent is a nucleic acid.

42. (original) The method of claim 41 wherein said liposome and said nucleic acid are present at a ratio ranging from 0.1-50 nanomole liposome per 1.0 µg nucleic acid.

43. (original) The method of claim 41 wherein said liposome and said nucleic acid are present at a ratio ranging from 1-24 nanomole liposome per 1.0 µg nucleic acid.

44. (original) The method of claim 41 wherein said liposome and said nucleic acid are present at a ratio ranging from 6-16 nanomole liposome per 1.0 µg nucleic acid.

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45. (original) The method of claim 40 wherein said complex has an acentric structure.

46. (original) The method of claim 45 wherein said complex has a solid core.

47. (currently amended) The method according to claim [39 or] 40, wherein said vector is administered systemically.

48. (currently amended) The method according to claim [39 or] 40, wherein said vector is administered intravenously.

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49. (currently amended) The method according to claim [39 or] 40, wherein the cell-targeting ligand is folate or transferrin, [the liposome is a cationic liposome] and the therapeutic agent is a nucleic acid encoding wild-type p53.

50. (currently amended) The method according to claim [39 or] 40 wherein the vector is administered in a pharmaceutically acceptable composition comprising a pharmaceutically acceptable vehicle.

51. (withdrawn)

52. (currently amended) A therapeutic method for the treatment or amelioration of cancer in a warm blooded animal, comprising administering to said animal a complex comprising a cancer cell targeting ligand, a cationic liposome comprising a cationic lipid and a neutral or helper lipid, and a therapeutic nucleic acid,

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wherein said complex has a mean diameter of less than about 100 nm
and the ligand is bound directly to the liposome.

53. (original) The method of claim 52 wherein said liposome and said nucleic acid are present at a ratio ranging from 0.1-50 nanomole liposome per 1.0 µg nucleic acid.

54. (original) The method of claim 53 wherein said liposome and said nucleic acid are present at a ratio ranging from 1-24 nanomole liposome per 1.0 µg nucleic acid.

55. (original) The method of claim 53 wherein said liposome and said nucleic acid are present at a ratio ranging from 6-16 nanomole liposome per 1.0 µg nucleic acid.

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56. (original) The method of claim 52 wherein said complex has an acentric structure.

57. (original) The method of claim 56 wherein said complex has a solid core.

58. (currently amended) The therapeutic method according to claim ~~51~~ or 52 wherein said complex is comprised of a cell-targeting ligand selected from the group consisting of folate and transferrin, a cationic liposome and a nucleic acid encoding wild-type p53.

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59. (original) The therapeutic method according to claim 58 wherein said complex is systemically administered to a cancer-bearing warm blooded animal.

60. (original) The therapeutic method according to claim 58, wherein said complex is intravenously administered to a cancer-bearing warm blooded animal.

61. (original) The therapeutic method according to claim 58, wherein said complex is intratumorally administered to a cancer-bearing warm blooded animal.

62. (original) The therapeutic method according to claim 58, further comprising administering an anti-cancer chemotherapeutic agent or an anti-cancer radiotherapy to said animal.

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63. (currently amended) A method for preparing complexes smaller than 100 nm in diameter wherein said complexes comprise a cationic liposome comprising [lipids] cationic lipid and a neutral or helper lipid, a ligand and a nucleic acid, said method comprising the steps of:

- a) mixing said ligand with said cationic liposome [lipids] to form a cationic liposome:ligand complex; and
- b) mixing said cationic liposome:ligand complex and said nucleic acid at a ratio of from 0.1-50 nanomoles liposome per 1.0 ug nucleic acid to form a cationic liposome:ligand:nucleic acid complex; [and
- c) rocking said liposome:ligand:nucleic acid complex.]

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wherein said cationic lipid comprises dioleoyltrimethylammonium-propane (DOTAP) or dimethyl dioctadecylammonium bromide (DDAB) and said neutral or helper lipid comprises dioleoylphosphatidylethanolamine (DOPE) or cholesterol.

64. (original) The method of claim 63 wherein said ratio is from 1-24 nanomoles liposome per 1.0 µg nucleic acid.

65. (original) The method of claim 63 wherein said ratio is from 6-16 nanomoles liposome per 1.0 µg nucleic acid.

66. (currently amended) The method of claim 63 wherein said [lipids comprise a] neutral lipid [selected from the group consisting of] comprises a combination of dioleoylphosphatidylethanolamine and cholesterol.

67. (currently amended) The method of claim 63 wherein said lipids comprise a combination of [cationic lipid selected from the group consisting of] DOTAP and [dimethyl dioctadecylammonium bromide] DOPE or cholesterol.

68. (original) The method of claim 63 wherein said ligand is folate or transferrin.

69. (currently amended) The method of claim 63 wherein said liposome:ligand complex of step (a) is incubated [with shaking] for 5-15 minutes before performing step (b).

70. (canceled herein)

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71. (new) The method of claim 63 wherein said lipids comprise a combination of DDAB and DOPE or cholesterol.

72. (new) The vector of claim 13, wherein said cationic lipid comprises DDAB or DOTAP.

73. (new) The vector of claim 13 or 72, wherein said neutral or helper lipid comprises DOPE or cholesterol.

74. (new) The vector of clam 31, wherein said cationic lipid comprises DOTAP or DDAB.

75. (new) The vector of claim 31 or 74, wherein said neutral or helper lipid comprises DOPE or cholesterol.

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76. (new) The method of claim 40, wherein said cationic lipid comprises DOTAP or DDAB.

77. (new) The method of claim 40 or 76, wherein said neutral or helper lipid comprises DOPE or cholesterol.

78. (new) The method of claim 52, wherein said cationic lipid comprises DOTAP or DDAB.

79. (new) The method of claim 52 or 78, wherein said neutral or helper lipid comprises DOPE or cholesterol.

80. (new) The method of claim 52, wherein said cancer comprises breast cancer, prostrate cancer, head and neck cancer,

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ovarian cancer, pancreatic cancer, colon cancer, glioblastoma, cervical cancer, lung cancer, gastric cancer, liposarcoma, melanoma and choriocarcinoma.

81. (new) The method of claim 52, wherein said cancer comprises breast cancer, prostate cancer, head and neck cancer, and pancreatic cancer.

82. (new) The method of claim 81, wherein said ligand comprises transferrin or folate, said therapeutic nucleic acid encodes wt p53 and said cationic liposome comprises a cationic lipid comprising DOTAP or DDAB and said neutral lipid comprises DOPE or cholesterol.

83. (new) The method of claim 63, which further comprises combining said complex with an aqueous solution of sucrose or dextrose.